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Bioanalytical method development and validation for simultaneous estimation of Teneligliptin and Rosuvastatin in plasma by RP-HPLC

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Abstract

A simple, precise, and robust reverse phase high-performance liquid chromatography (RP HPLC) method was developed and validated for the simultaneous estimation of Teneligliptin (TGL) and Rosuvastatin (RVS) in plasma. Chromatographic separation was achieved using a C18 column (4.6 × 250 mm, 5 µm) with a mobile phase of water–acetonitrile (50:50, pH 2.8 adjusted with formic acid) at a flow rate of 1.2 mL/min. Detection was performed at 265 nm with a run time of 10 minutes. The retention times of TGL and RVS were 2.464 min and 7.733 min, respectively. The method was validated according to ICH Q2(R1) guidelines for system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Linearity was observed in the range of 6–16 µg/mL for both drugs, with correlation coefficients of 0.9956 (TGL) and 0.9913 (RVS). Recovery studies demonstrated mean recoveries of 98.31% for TGL and 98.86% for RVS, confirming accuracy. Precision studies showed %RSD values below 0.2%, indicating excellent reproducibility. The LOD and LOQ were 0.92 µg/mL and 2.79 µg/mL for TGL, and 1.29 µg/mL and 3.91 µg/mL for RVS, respectively. The method proved to be specific, accurate, and reliable for bioanalytical applications, making it suitable for pharmacokinetic and clinical research studies.

Keywords: Bioanalytical, plasma, Teneligliptin, rosuvastatin, RP-HPLC

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1. Introduction

Teneligliptin (TGL) is a novel, potent dipeptidyl peptidase-4 (DPP-4) inhibitor widely used in the management of type 2 diabetes mellitus. By inhibiting DPP-4, Teneligliptin prolongs the activity of incretin hormones such as glucagon-like peptide-1 (GLP-1), thereby enhancing insulin secretion and suppressing glucagon release in a glucose-dependent manner, ultimately improving glycemic control [1,2]. Rosuvastatin (RVS), on the other hand, is a synthetic lipid-lowering agent belonging to the class of HMG-CoA reductase inhibitors (statins). It competitively inhibits the conversion of HMG-CoA to mevalonate, a precursor of cholesterol, leading to reduced hepatic cholesterol synthesis and increased clearance of low-density lipoprotein (LDL) cholesterol [3,4].

Patients with type 2 diabetes often present with dyslipidemia, characterized by elevated triglycerides and LDL cholesterol, which significantly increases cardiovascular risk. The fixed-dose combination of Teneligliptin and Rosuvastatin addresses both hyperglycemia and dyslipidemia, offering a synergistic therapeutic approach for diabetic patients with comorbid hyperlipidemia [5]. This dual therapy reduces the burden of polypharmacy and enhances patient compliance.

Several analytical methods have been reported for the individual estimation of Teneligliptin and Rosuvastatin, including spectrophotometry, LC-MS, and HPTLC [6,7]. However, no study was found to describe simultaneous estimation of both drugs, particularly in plasma, which is essential for pharmacokinetic and bioequivalence studies [8]. Reverse phase high-performance liquid chromatography (RP-HPLC) remains the method of choice due to its sensitivity, reproducibility, and ability to separate analytes with high resolution under optimized conditions [9].

The present study aims to develop and validate a simple, precise, and robust RP-HPLC bioanalytical method for simultaneous estimation of Teneligliptin and Rosuvastatin in plasma. The method was validated according to ICH Q2(R1) guidelines for parameters including accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness. Such a validated

method is expected to be highly useful for routine quality control, pharmacokinetic profiling, and clinical research applications [10].

2. Material and Methods

Teneligliptin (TGL) (>98%) and Rosuvastatin (RVS) (>98%) pure drugs were purchased from Yarrow Pharmaceuticals, Mumbai. Plasma was procured from Bhopal Memorial Hospital and Research Center; all solvents used were of HPLC grade.

Instrument Used for method development

A Shimadzu HPLC system (P-series) equipped with PDA detector (SPD-M40), pump (LC-20AD), column oven (CTO-10AS VP), C-18 column (Shim-pack Solar, 4.6/250 mm, 5 μ) and autosampler (SIL-20AC HT) was used for the study.

Preparation of standard solution

A mixed standard stock solution of TGL and RVS was prepared by dissolving 10 mg each of both the drugs in 10 mL methanol. 2.0 mL of this stock solution was withdrawn and diluted with plasma (8 mL) to obtain a solution containing 200 μ g/mL of TGL and RVS. 1.0 mL of the above solution was further diluted with plasma to produce working standard solution containing TGL (20 μ g/mL) and RVS (20 μ g/mL). The solution was sonicated for 10 min and filtered through 0.45 μ membrane filter before use.

Extraction of sample

Take 200 μ L of blank plasma and add RSV working standard to it. Add to it 500 μ L of ice-cold acetonitrile/ethylacetate and vortex for 3 min. The solution was centrifuged at 10000 rpm for 10 min and the clear supernatant was collected. The organic layer was separated, evaporated at room temperature and reconstituted with 200 μ L of the mobile phase. This solution was injected in the HPLC system and analyzed using the optimized parameters.

Optimized conditions

The conditions for the simultaneous estimation of TGL and RVS include:

Column: Octadecylsilane (C₁₈) (4.6 x 250mm, 5μm, Shim-pack Solar)

Mobile Phase –Water-acetonitrile (50:50), pH adjusted to 2.80 with formic acid

Flow rate: 1.2 ml per min

Wavelength: 265 nm

Injection volume: 20 μl

Column Temperature: 40°C

Run time: 10 min

Validation of method

The method was validated according to ICH Q2(R1) guidelines for accuracy, precision, linearity, limit of detection, limit of quantification and robustness [11,12].

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Specificity

Solutions of standard and samples were prepared as per test procedure and injected into the HPLC system. The chromatograms were recorded. A study to establish the interference of blank was conducted by injected the mobile phase into HPLC system.

Linearity

The working standards were prepared at different concentrations by diluting with the diluents. The dilutions were injected in to the HPLC system and analyzed as per the optimized conditions.

Accuracy

Accuracy of the method was determined by recovery studies. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at two different levels (50%, and 100%) of the target assay concentration. The amounts added, amounts estimated and the individual recovery and mean recovery values were calculated

Precision

Repeatability

The working standard solution was injected in six replicates in the HPLC system and the peak area was measured. The % RSD for the area was calculated.

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was evaluated on different days by different analysts.

Limit of Detection and quantification

The LOD and LOQ were calculated using calibration curve method. The following formula was used.

$$\text{LOD} = 3.3 \text{ sigma}/S$$

$$\text{LOQ} = 10 \text{ sigma}/S$$

Where sigma is standard deviation of the calibration curve, S is the slope of the calibration curve

Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous batches by differing physical parameters like flow rate and mobile phase composition which may differ but the responses would be still within the specified limits of the assay.

3. Results and Discussion

Method Development

The optimum chromatographic conditions were selected on the basis our previous work for

simultaneous estimation of both the drugs in bulk and dosage form [13].

The chromatographic conditions were detection wavelength of 265 nm, injection volume of 20 μ L, flow rate of 1.2 mL/min, column temperature of 40°C, a C18 column of 25 cm length with internal diameter of 4.6 mm and particle size of 5 μ m. The mobile phase comprised of acetonitrile-water (pH 2.8, adjusted with formic acid). The chromatogram exhibited elution of teneligliptin and rosuvastatin at 2.464 and 7.733 min respectively (Figure 1).

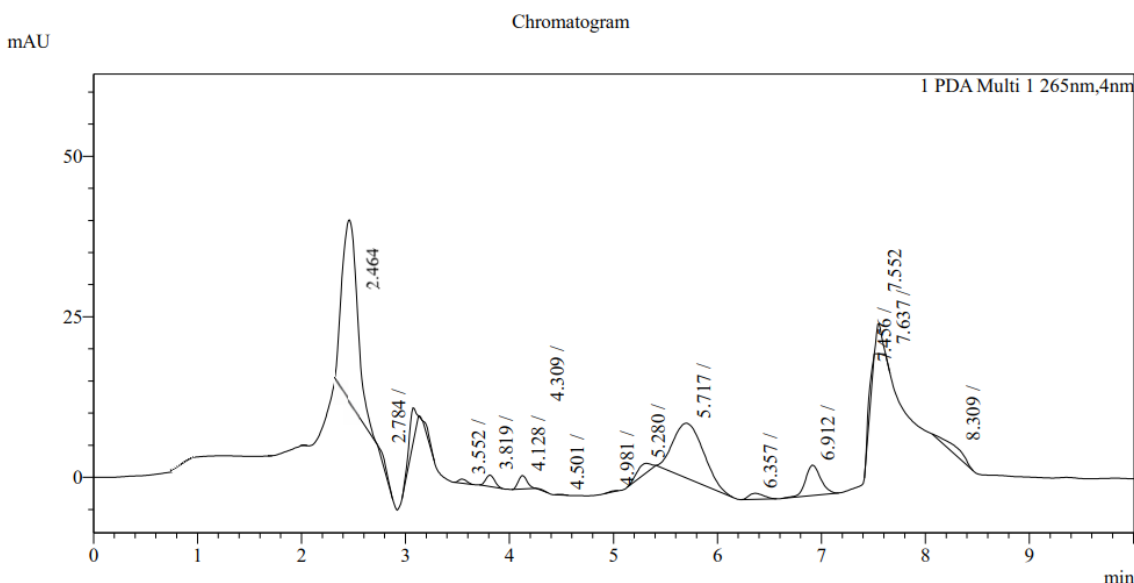


Figure 1. Chromatogram of TGL and RVS in plasma

Validation of the method

The validation of the analytical method is done to assure that the proposed method will be consistently deliver the accurate results. The ICH guidelines were used to validate the method and parameters like system suitability, accuracy, precision, robustness and linearity were studied.

System suitability

System suitability evaluation provides a harmony that the equipment, electronics, analytical operations and sample as a whole are integral part of the analysis and are suitable for the analysis. The system suitability results obtained are presented in Table 1 and 2.

Table 1. Results of system suitability study for teneligliptin

System suitability parameters	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Mean	SD	RSD
Peak Area	79321	79408	79347	79389	79353	79391	79368.17	34.710	0.044
Retention Time	2.464	2.433	2.464	2.451	2.464	2.368	2.441	0.014	0.558
No. of Theoretical plates	8459	8465	8678	8466	8688	8428	8530.67	120.40	-
Tailing Factor	1.363	1.225	1.296	1.192	0.617	1.276	1.162	0.299	-

The retention times for both analytes are highly reproducible, as indicated by the very low RSD values (<0.2%). This demonstrates excellent precision of the chromatographic system. The narrow standard deviations confirm stability in column performance and mobile phase composition. The tailing factor for both drugs is close to 1, which indicates symmetrical peak shapes. Teneligliptin shows a slight tailing (1.27), but still within acceptable pharmacopeial limits (<2). Rosuvastatin peaks are nearly symmetrical confirming minimal peak distortion and efficient interaction between analyte and stationary phase. Theoretical plate numbers reflect column efficiency. Rosuvastatin shows an exceptionally high plate count, indicating sharp, well-resolved peaks and excellent separation efficiency. Teneligliptin's plate count is lower but still acceptable for small molecules, suggesting adequate resolution for its retention window. The system suitability parameters confirm that the developed RP-HPLC method is precise, efficient, and suitable for quantitative analysis of Teneligliptin

and Rosuvastatin. The method can be confidently applied for routine quality control and research applications.

Table 2. Results of system suitability study for rosuvastatin

System suitability parameter s	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	Mean	SD	RSD
Peak Area	16537	16628	16509	16711	16623	16539	16591.17	80.423	0.485
Retention Time	7.733	7.733	7.669	7.627	7.552	7.553	7.645	0.077	1.002
No. of Theoretical plates	112713	115774	115138	116064	115716	115238	115107.2	1365.69	-
Tailing Factor	1.241	1.268	2.099	1.296	1.348	2.188	1.573	0.365	-

Specificity

Specificity indicates the ability of the method to particularly detect and quantify the analyte of interest in the presence of various other components that might be present in the solution. An injection of blank mobile phase was analysed using the developed method for assessing any possible interference in analysis of teneligliptin and rosuvastatin by the method (Figure 2).

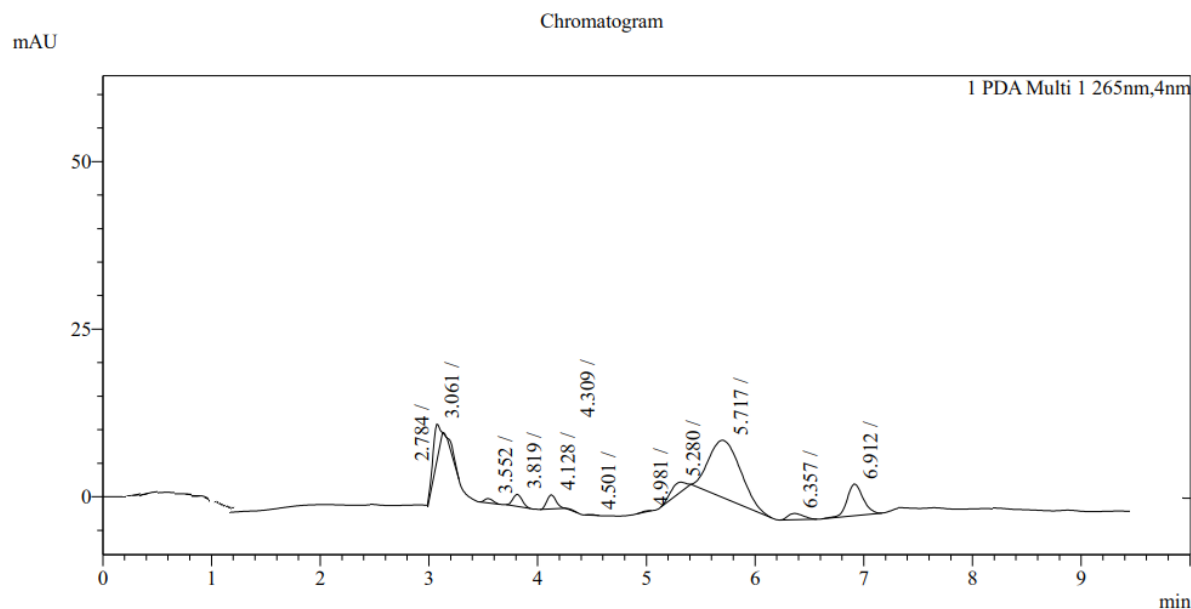


Figure 2. Chromatogram of blank (plasma)

The clear separation of Teneligliptin and Rosuvastatin peaks, along with the absence of interference from excipients or other components, demonstrates that the method is specific. The use of a PDA detector at 265 nm further supports spectral purity, ensuring that each peak corresponds to a single component.

Precision

Precision portrays the capability of the method to yield the same results regardless of the instrument used, or day of analysis or the analyst executing the analysis. Precision is expressed as the % RSD of the several replicate analyses of the same sample. The results of intra-day (repeatability) and inter-day (ruggedness) precision are reported in Table 3 and 4 respectively. Both repeatability (%RSD 0.068 and 0.035 for Teneligliptin and Rosuvastatin respectively) and intermediate precision (%RSD 0.075 and 0.021 for Teneligliptin and Rosuvastatin respectively) results satisfy ICH Q2(R1) criteria (<2%RSD). The close agreement between mean values and low standard deviations indicate that the method provides reliable quantitative results for Teneligliptin and Rosuvastatin. The chromatographic system exhibits stable retention behaviour and uniform detector response, confirming the precision of

the developed method.

Table 3. Repeatability of estimation using proposed method

	Teneligliptin		Rosuvastatin	
	RT	Area	RT	Area
Rep 1	2.461	152647	7.739	30974
Rep 2	2.464	153122	7.735	31033
Rep 3	2.464	152603	7.733	30996
Rep 4	2.465	153028	7.733	31015
Rep 5	2.461	152629	7.739	31121
Rep 6	2.463	152538	7.736	31136
Mean	2.463	152761.2	7.735833	31045.83
SD	0.002	247.678	0.003	67.140
RSD	0.068	0.162	0.035	0.216

Table 4. Ruggedness (Intermediate Precision) of estimation using proposed method

	Teneligliptin		Rosuvastatin	
	RT	Area	RT	Area
Rep 1	2.46	153129	7.733	30994
Rep 2	2.461	152597	7.736	31124
Rep 3	2.465	152623	7.736	30986
Rep 4	2.461	152629	7.735	31075
Rep 5	2.463	152588	7.738	31113
Rep 6	2.463	152627	7.735	31086

Mean	2.462	152698.8	7.736	31063
SD	0.002	211.417	0.002	59.302
RSD	0.075	0.138	0.021	0.191

Linearity and Range

For estimation of teneligliptin, the calibration range was found to be 6 to 16 µg/ml (Table 5, Figure 3). The equation for calibration curve was found to be having a regression coefficient of 0.9956 with slope of 11816 and intercept of 6272.3.

For estimation of rosuvastatin, the calibration on the other hand was found to be 6 to 16 µg/ml. The equation for calibration curve was found to be having a regression coefficient of 0.9913 with slope of 2362.6 and intercept of 1677.7.

Table 5. Linearity and range data

Concentration (µg/ml)	Area under curve of Teneligliptin	Area under curve of Rosuvastatin
6	79353	16537
8	97228	19342
10	123101	25412
12	152647	30974
14	169883	34065
16	195274	39667
18	260997	49353
24	274533	54869
30	286360	62737

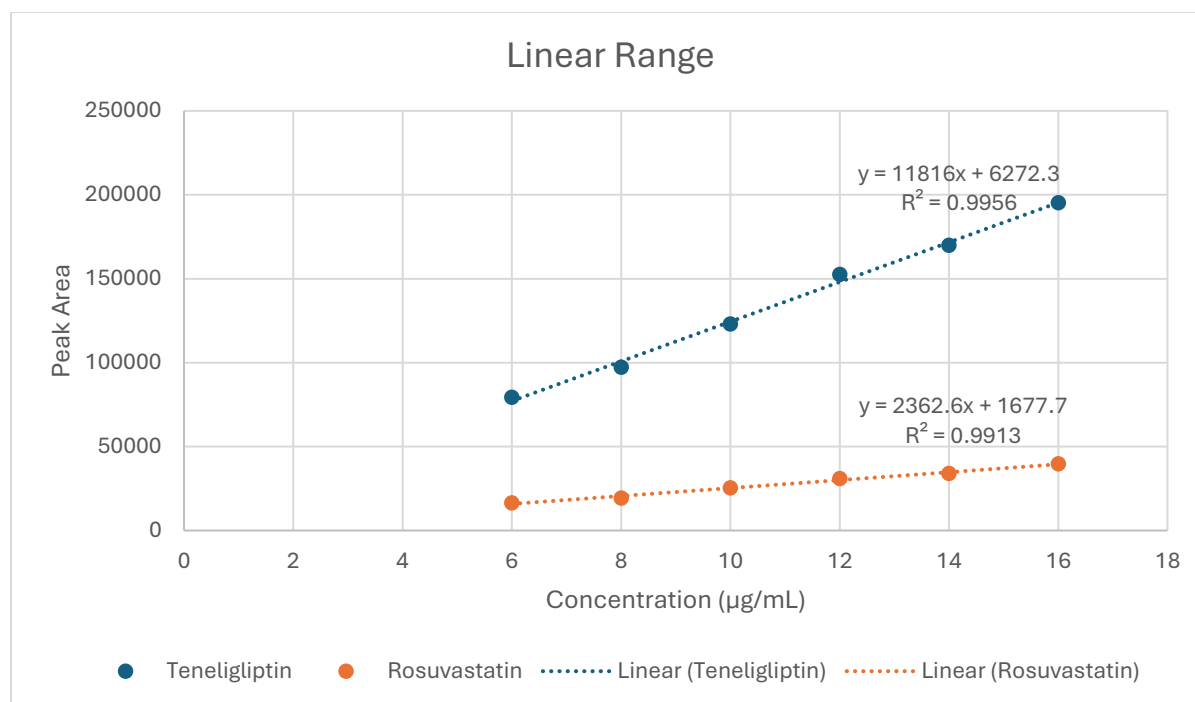


Figure 3. Calibration range of the proposed method

Although the detector responds up to 30 µg/mL, the non-linear behaviour beyond 16 µg/mL restricts the quantitative application to the lower range. This ensures accurate, precise, and reproducible quantification of both analytes within the validated limits.

Accuracy

The accuracy of an analytical method articulates the closeness of agreement between the values which is accepted either as a conventional true value or an accepted reference value for the observed value.

The accuracy of method is determined by performing the recovery studies that involve spiking of a pre-analyzed sample with known amount of standard and re-analyzing it to calculate the amount of added sample (recovery).

The spiked samples of teneligliptin were recovered in range of 98.28 to 98.33 % suggesting an accuracy in determining the spiked samples (Table 6). The mean recovery of the teneligliptin from the spiked samples was 98.31 ± 0.775 %.

Table 6. Accuracy results for the proposed method for teneligliptin

Conc. of drug in sample $\mu\text{g/ml}$	Conc. of drug added to final (μg)	Amount Recovered ($\mu\text{g/ml}$)			Mean Recovery	% Recovered (mean)
		Rep 1	Rep 2	Rep 3		
6	3	8.94	8.72	8.89	8.85	98.33
6	6	11.83	11.77	11.78	11.793	98.28
Mean						98.31
SD						0.775

Table 7. Accuracy results for the proposed method for rosuvastatin

Conc. of drug in sample $\mu\text{g/ml}$	Conc. of drug added to final (μg)	Amount Recovered ($\mu\text{g/ml}$)			Mean Recovery	% Recovered (mean)
		Rep 1	Rep 2	Rep 3		
6	3	8.9	8.91	8.91	8.906	98.96
6	6	11.89	11.76	11.9	11.85	98.75
Mean						98.86
SD						0.356

The spiked samples of rosuvastatin were recovered in range of 98.96 to 98.75 % suggesting an accuracy in determining the spiked samples (Table 7). The mean recovery of the rosuvastatin from the spiked samples was 98.96 ± 0.356 %.

The accuracy study confirms that the developed RP-HPLC method is accurate and reliable for simultaneous estimation of Teneligliptin and Rosuvastatin. The recovery values (98–99%) and low variability meet ICH Q2(R1) acceptance criteria, validating the method for quantitative analysis in pharmaceutical formulations.

Robustness

The robustness of a method is the ability of method to stay unaffected by small, measured variations in the method parameters and delivers a suggestion of method dependability and consistency during regular use.

A few deliberate changes in flow rate and mobile phase composition were done to study its effect on the results obtained by the proposed method. The method was able to adjust to the changes with no significant change in the retention time of the eluted components. The results are presented in Tables 8 and 9.

Table 8. Effect of change in flow rate

Flow rate	Teneligliptin		Rosuvastatin	
	Retention Time	Peak Area	Retention Time	Peak Area
1	2.639	153122	8.309	30996
1.5	2.251	152603	7.456	31015

Table 9. Effect of change in mobile phase composition

Mobile Phase composition	Teneligliptin		Rosuvastatin	
	Retention Time	Peak Area	Retention Time	Peak Area
60FA:40ACN	2.433	152647	7.329	31121
40FA:60ACN	2.457	152538	7.336	31136

The method consistently produced identifiable peaks for both analytes under deliberate variations in flow rate and mobile phase composition. Retention times shifted predictably, but resolution between Teneligliptin and Rosuvastatin remained acceptable. Peak areas varied, reflecting sensitivity to detector response and elution strength, but quantification remained possible within acceptable limits.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the calibration curve method. The LOD and LOQ was found to be 0.92 µg/mL and 2.79 µg/mL for teneligliptin and 1.29 µg/mL and 3.91 µg/mL for rosuvastatin.

4. Conclusion

The objective of our study was to develop and validate a simple, yet accurate, reproducible and robust method for simultaneous estimation of Teneligliptin and Rosuvastatin in plasma samples. The developed RP-HPLC method successfully enabled the simultaneous estimation of Teneligliptin and Rosuvastatin in plasma with high sensitivity, reproducibility, and accuracy. Validation studies confirmed compliance with ICH Q2(R1) guidelines, demonstrating excellent system suitability, specificity, precision, linearity, and robustness. The recovery values (98–99%) and low %RSD values highlight the reliability of the method for quantitative analysis. Furthermore, the low LOD and LOQ values establish the method's applicability for trace level detection in biological samples. Overall, this validated RP-HPLC method can be confidently applied for routine quality control, pharmacokinetic profiling, and bioequivalence studies of Teneligliptin and Rosuvastatin in plasma, thereby supporting

both clinical and pharmaceutical research.

Conflict of Interest

The author declares that there are no conflicts of interest associated with this research. The author has no financial, personal, or professional relationships that could have influenced the design, analysis, interpretation, or reporting of the findings presented in this manuscript.

Author Contributions

Conceptualization, methodology development, data analysis, and machine learning framework design was performed by the authors. Manuscript preparation, visualization, and interpretation of results was carried out by the Abhilasha Kumari. Authors reviewed, revised, and approved the final version of the manuscript.

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